BRC Science Highlight
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TMTpro Complementary Ion Quantification Increases Plexing and Sensitivity for Accurate Multiplexed Proteomics at the MS2 Level

Background/Objective

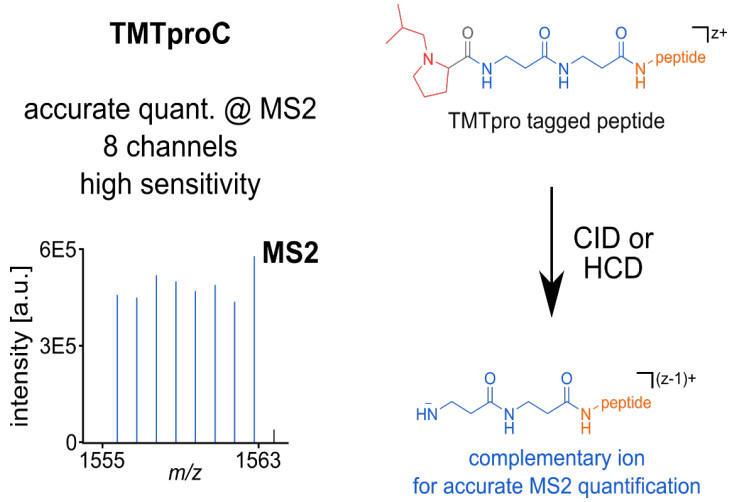
Quantitative proteomics is a powerful tool to analyze changes in protein concentrations across multiple biological samples of nearly any origin. Using isobaric tags, multiple protein samples can be quantified simultaneously, increasing sample throughput, measurement reproducibility, and measurement precision. The state-of-the-art MS3-based methods improve quantification accuracy of relative protein changes but reduce sensitivity and require specialized instrumentation. Quantification by complementary ions is an alternative approach, but the popular isobaric TMT tag limits multiplexing to five channels and complementary ions form inefficiently. Here, we evaluate TMTproC, a complementary ion quantification method optimized for use with a new isobaric tag TMTpro. We show that TMTproC increases plexing capacity to eight channels and the favorable chemical properties of TMTpro results in ~65% more proteins quantified than MS3 quantification.

Approach

- Proteins from multiple samples are enzymatically digested, and the resulting peptides are labeled with isobaric tags. The samples are then combined before analysis on a mass spectrometer in a single run.
- Yeast peptides from *Saccharomyces cerevisiae* lysates were used for method optimization.
- Method efficacy was evaluated by accurately quantifying yeast peptides in a complex background.

Results

- TMTproC increases throughput and experimental flexibility by increasing the maximum number of simultaneously analyzed samples to eight.
- The beneficial fragmentation properties of TMTpro increase sensitivity for TMTproC, resulting in ~65% more proteins quantified compared to TMTpro-MS3 and ~18% more compared to real-time-search TMTpro-MS3.
- TMTproC reduces interference more than TMTpro-MS3, improving measurement accuracy even further.
- The software for quantifying TMTproC data is provided as an executable that is compatible with the MaxQuant analysis pipeline, widening access to accurate multiplexed proteomics.



Accurate and sensitive proteomic analysis is more accessible with TMTproC.

Significance

TMTproC advances multiplexed proteomics data quality and widens access to accurate multiplexed proteomics beyond laboratories with MS3-capable instrumentation. The methods inherent flexibility makes it the ideal tool for proteomic analysis in a wide variety of bioenergy applications.

Johnson, A., et al. 2021. "TMTpro Complementary Ion Quantification Increases Plexing and Sensitivity for Accurate Multiplexed Proteomics at the MS2 Level." Journal of Proteome Research (20) 6: 3043–3052. DOI: 10.1021/acs.jproteome.0c00813.

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